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<p>(54) Title: SYNERGISTS OF BACILLUS THURINGIENSIS DELTA-ENDOTOXIN</p> <p>(57) Abstract</p> <p>The present invention relates to pesticidal compositions comprising an amount of a <i>Bacillus thuringiensis</i> biopesticide and a synergist in an effective amount for enhancing the pesticidal activity of the <i>Bacillus thuringiensis</i> biopesticide for controlling the pest or for reducing the amount of the <i>Bacillus thuringiensis</i> biopesticide typically needed to be a commercially effective biopesticide. The present invention further relates to a method for controlling a pest comprising exposing the pest to said pesticidal compositions of the present invention.</p>		

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## SYNERGISTS OF *BACILLUS THURINGIENSIS* DELTA-ENDOTOXIN

### Background of the Invention

#### Field of the Invention

The present invention relates to pesticidal compositions comprising a *Bacillus thuringiensis* biopesticide and a synergist, wherein the synergist enhances the pesticidal activity of a *Bacillus thuringiensis* biopesticide and/or reduces the amount of the *Bacillus thuringiensis* biopesticide typically needed to be a commercially effective biopesticide.

#### Description of the Related Art

*Bacillus thuringiensis* is a motile, rod-shaped, gram-positive bacterium that is widely distributed in nature, especially in soil and insect-rich environments. During sporulation, *Bacillus thuringiensis* produces a parasporal crystal inclusion(s) which is insecticidal upon ingestion to susceptible insect larvae of the order Lepidoptera, Diptera, or Coleoptera. The inclusions may vary in shape, number, and composition. They are comprised of one or more proteins called delta-endotoxin proteins, which may range in size from 27-140 kDa. The insecticidal delta-endotoxin proteins are generally converted by proteases in the larval gut into smaller (truncated) toxic polypeptides, causing midgut destruction, and ultimately, death of the insect.

*Bacillus thuringiensis* crystal delta-endotoxin proteins are the most widely used biopesticide. There are several *Bacillus thuringiensis* strains that are used as producers of crystal delta-endotoxin proteins for the forestry, agricultural, and public health areas. *Bacillus thuringiensis* subsp. *kurstaki* and *Bacillus thuringiensis* subsp. *aizawai* produce delta-endotoxin proteins specific for Lepidoptera. A delta-endotoxin protein specific for Coleoptera is produced by *Bacillus thuringiensis* subsp. *tenebrionis*. Furthermore, *Bacillus thuringiensis* subsp. *israelensis* produces delta-endotoxin proteins specific for Diptera.

Several *Bacillus thuringiensis* crystal delta-endotoxin proteins are also reportedly pesticidal to nematodes, Acari, Hymenoptera, Phthiraptera, Platyhelminthes, Homoptera, Blattodea, and Protozoa.

The delta-endotoxin proteins are encoded by *cry* (crystal protein) genes which are generally located on plasmids. The *cry* genes have been divided into six classes and several subclasses based on relative amino acid homology and pesticidal specificity. The major classes are Lepidoptera-specific (*cryI*); Lepidoptera- and Diptera-specific (*cryII*); Coleoptera-specific (*cryIII*); Diptera-specific (*cryIV*); Coleoptera- and Lepidoptera-specific (*cryV* genes); and Nematode-specific (*cryVI* genes).

The art has strived to improve the effectiveness and to broaden the host range of *Bacillus thuringiensis* delta-endotoxin proteins. Efforts have been directed to increasing the pesticidal activity of *Bacillus thuringiensis* biopesticides by combining the biopesticide with a compound that enhances the pesticidal activity. Burgess discloses that p-aminosalicylic acid enhances the pesticidal activity of a *Bacillus thuringiensis* subsp. *berliner* biopesticide against *Galleria mellonella* larvae (Burgess, 1977, *Apidologie* 8:155-168). Salama *et al.* disclose that calcium carbonate, potassium carbonate, potassium dicarbonate, potassium dibasic phosphate, zinc sulfate, ammonium dibasic phosphate, acetamide, L-valine, L-arginine, picric acid, and disodium 2-glycerophosphate increases the pesticidal activity of a *Bacillus thuringiensis* subsp. *kurstaki* biopesticide against *Spodoptera littoralis* larvae (Salama *et al.*, 1986, *Journal of Applied Entomology* 101, 304-313). Hellpap and Zebitz disclose that the combination of Neem (azadirachtin) and a *Bacillus thuringiensis* subsp. *kurstaki* biopesticide against 4 day old *Spodoptera frugiperda* larvae is mainly additive at levels where individually Neem (25 to 125 ppm) and the biopesticide (0.5%) are lethal, but show some synergism when Neem is present at levels between 25 and 125 ppm with the biopesticide at 0.1% (Hellpap and Zebitz, 1986, *Journal of Applied Entomology* 101:515-524). Antagonism is observed when Neem is present at a low level of 25 ppm with the biopesticide at 0.5%. However, Moar and Trumble disclose that there is no synergistic interaction of Neem with a biopesticide from *Bacillus thuringiensis* subsp. *kurstaki* against *Spodoptera exigua* larvae, but rather an antagonistic effect (Moar and Trumble, 1987, *Journal of Economic Entomology* 80:588-592). Knauf *et al.* disclose the synergistic combination of avermectin and a *Bacillus thuringiensis* biopesticide (Knauf *et al.*, 1987, European Patent Application No. 242 502 A2). Noriyoshi and Hisaki disclose a synergistic composition containing avermectin B1 and a *Bacillus thuringiensis* biopesticide (1984, JP 59199616). Guertin *et al.* disclose the use of a phagostimulant to enhance the pesticidal activity of *Bacillus thuringiensis* biopesticides (Guertin *et al.*, 1994, European Patent Application No. 598 156 A1). Finally, Morris *et al.*

disclose that caffeine increases the pesticidal activity of a *Bacillus thuringiensis* subsp. *kurstaki* biopesticide against *Mamestra configurata* larvae (Morris *et al.*, 1994, *Journal of Economic Entomology* 87:610-617).

It is an object of the present invention to provide improved pesticidal compositions, comprising a synergist and a *Bacillus thuringiensis* biopesticide, with enhanced pesticidal activity. It is a further object of the present invention to provide a method for controlling pest infestation with the compositions.

### Summary of the Invention

The present invention relates to pesticidal compositions comprising an amount of a *Bacillus thuringiensis* biopesticide and a synergist in an effective amount for enhancing the pesticidal activity of the *Bacillus thuringiensis* biopesticide and/or for reducing the amount of the *Bacillus thuringiensis* biopesticide typically needed to be a commercially effective biopesticide. The present invention further relates to a method for controlling pest infestation on plants comprising applying to plants the pesticidal compositions.

### Detailed Description of the Invention

It has now been unexpectedly found that certain classes of compounds function as synergists capable of enhancing the pesticidal activity of a *Bacillus thuringiensis* biopesticide, and, therefore, permits the use of smaller amounts of the *Bacillus thuringiensis* biopesticide than would typically be needed to achieve commercially acceptable control of a pest or improves the efficacy of a *Bacillus thuringiensis* biopesticide which alone would have been commercially impractical or commercially ineffective as a biopesticide.

The present invention is directed to pesticidal compositions comprising an amount of a *Bacillus thuringiensis* biopesticide and a synergist in an effective amount for enhancing the pesticidal activity of the *Bacillus thuringiensis* biopesticide to effectively control the pest. "Pesticidal activity" is defined herein as a measure of the amount of activity of a *Bacillus thuringiensis* biopesticide against a pest through killing or stunting of the growth of the pest or protecting a plant from pest infestation. "Ineffective amount" is defined herein as the amount of a *Bacillus thuringiensis* biopesticide which provides less than about 35% control of a pest when used alone. "Effective amount" is defined herein as the amount of a synergist sufficient to enhance the pesticidal activity of a *Bacillus thuringiensis* biopesticide

by about 20%, preferably by about 40%, more preferably by about 60%, and most preferably by about 100%. The synergist may or may not have pesticidal activity at the rates used to synergize the pesticidal activity of a *Bacillus thuringiensis* biopesticide.

The pesticidal activity of a *Bacillus thuringiensis* biopesticide and the synergistic effect of a synergist on the pesticidal activity of a *Bacillus thuringiensis* biopesticide may be assayed using procedures known in the art, such as artificial diet incorporation, artificial diet overlay, leaf painting, leaf dip, and foliar spray.

A *Bacillus thuringiensis* biopesticide alone, at certain levels, may provide commercially acceptable control of a pest. However, the amount of any given *Bacillus thuringiensis* biopesticide required to achieve commercially acceptable control may be quite high, and, therefore, impractical economically. In accordance with the present invention, however, the amount of a *Bacillus thuringiensis* biopesticide required in any given composition may be reduced by combining the *Bacillus thuringiensis* biopesticide with an effective amount of a synergist to enhance the pesticidal activity of the *Bacillus thuringiensis* biopesticide.

The amount of a *Bacillus thuringiensis* biopesticide needed to achieve commercially acceptable control of a pest will differ from one *Bacillus thuringiensis* biopesticide to another, and furthermore, will vary depending on the pest and the crop. The efficacy of a large number of *Bacillus thuringiensis* biopesticides is well established in the art. The amounts required for commercial utility are either known or are easily determined by the ordinary skilled artisan. For example, in agriculture in the treatment of lettuce, an amount of a *Bacillus thuringiensis* biopesticide required for commercial utility is that which achieves greater than about 80% control of *Spodoptera exigua*. In the treatment of potato infestations late in the season, an amount of a *Bacillus thuringiensis* biopesticide adequate to achieve about 35% control of *Leptinotarsa decimlineata* is considered to be effective. In forestry, where protection against defoliation is needed, an amount of a *Bacillus thuringiensis* biopesticide which achieves about 50% control of *Lymantria dispar* is considered to be effective.

The benefit of the present invention may be achieved by determining the amount of a *Bacillus thuringiensis* biopesticide required alone to achieve commercially acceptable control of a pest. The amount of a synergist needed to provide the benefit of reducing the amount of a *Bacillus thuringiensis* biopesticide used commercially can be

determined, for example, by taking the amount of a *Bacillus thuringiensis* biopesticide required to achieve commercially acceptable control of a pest and progressively reducing that amount of the *Bacillus thuringiensis* biopesticide, e.g., 10%, and progressively adding a synergist in increasing amounts until the level of control of the pest equals that which is achieved with the *Bacillus thuringiensis* biopesticide alone in amounts typically used commercially.

Likewise, the combination of a *Bacillus thuringiensis* biopesticide and a synergist may be useful in bringing about the commercial use of the *Bacillus thuringiensis* biopesticide which alone would have been commercially impractical or commercially ineffective as a biopesticide.

Several classes of synergists have been determined to enhance the pesticidal activity of a *Bacillus thuringiensis* biopesticide. In a preferred embodiment, the synergist is an azadirachtin, e.g., Neem seed extract, azadirachtin A, azadirachtin B, or derivatives and analogs of an azadirachtin.

In another preferred embodiment, the synergist is zinc oxide or zinc chloride.

In another preferred embodiment, the synergist is a combination of azadirachtin and a zinc salt. In a more preferred embodiment, the synergist is a combination of azadirachtin and zinc oxide.

In another preferred embodiment, the synergist is an amphiphatic lipid wherein said amphiphatic lipid is decanoyl-N-methyl-glucamide, digalactosyl diglyceride (glycerol moiety esterified with oleic acid and palmitic acid), dodecyl glucopyranoside, dodecyl trimethyl ammonium bromide, triolein, or phosphatidylethanolamine.

In another preferred embodiment, the synergist is an analgesic wherein said analgesic is acetylsalicylic acid, ibuprofen, or acetaminophen.

In another preferred embodiment, the synergist is esculin.

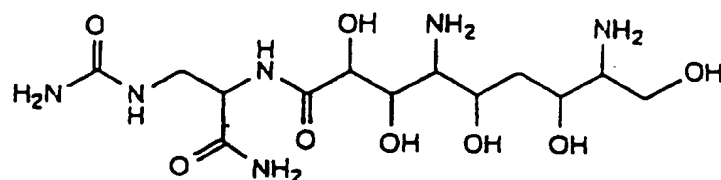
In another preferred embodiment, the synergist is poly-L-lysine.

In another preferred embodiment, the synergist is alpha-tocopherol.

The synergists disclosed above may be obtained synthetically or from natural sources.

Also within the scope of the present invention, the pesticidal compositions of the present invention may comprise a combination of two or more of the synergists described above. Furthermore, the pesticidal compositions of the present invention may comprise one

or more synergists, potentiators, or phagostimulants known in the art. For example, the potentiator disclosed in WO 94/09630 or a pesticidally-active salt thereof may be used in the compositions of the present invention:



The compositions of the present invention comprise the synergist in an amount between about 0.001 and about 100 grams, preferably between about 0.001 and about 50 grams, more preferably between about 0.001 and about 25 grams, more preferably between about 0.001 and about 10 grams, more preferably between about 0.001 and about 2 grams, more preferably between about 0.001 and about 0.2 gram, more preferably between about 0.001 and about 0.1 gram, and most preferably between about 0.001 and about 0.01 gram per gram of *Bacillus thuringiensis* biopesticide. These amounts are based on a composition in which the biopesticide is a *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate, where a portion of the water and solubles have been removed from the whole broth by centrifugation and preserved at an acid pH of 4.3 to 5.5, with a potency of 8000 IU (International Units) per mg solids (bioassayed against 3rd instar *Trichoplusia ni* using the *Bacillus thuringiensis* subsp. *kurstaki* standard HD1-S-1980, USDA, Peoria, Illinois). It will be obvious to a person skilled in the art that the amount of a particular synergist preparation needed to enhance a *Bacillus thuringiensis* biopesticide may vary significantly depending on the purity of the synergist preparation.

The *Bacillus thuringiensis* biopesticide in the pesticidal compositions of the present invention may be derived from, but not limited to, *Bacillus thuringiensis* subsp. *aizawai*, *Bacillus thuringiensis* subsp. *alesti*, *Bacillus thuringiensis* subsp. *canadiensis*, *Bacillus thuringiensis* subsp. *colmeri*, *Bacillus thuringiensis* subsp. *coreanensis*, *Bacillus thuringiensis* subsp. *dakota*, *Bacillus thuringiensis* subsp. *darmstadiensis*, *Bacillus thuringiensis* subsp. *dendrolimus*, *Bacillus thuringiensis* subsp. *entomocidus*, *Bacillus thuringiensis* subsp. *finitimus*, *Bacillus thuringiensis* subsp. *galleriae*, *Bacillus thuringiensis* subsp. *indiana*, *Bacillus thuringiensis* subsp. *israelensis*, *Bacillus thuringiensis* subsp. *kenyae*, *Bacillus thuringiensis* subsp. *kumamotoensis*, *Bacillus thuringiensis* subsp. *kurstaki*, *Bacillus*



*thuringiensis* subsp. *kyushuensis*, *Bacillus thuringiensis* subsp. *japonensis*, *Bacillus thuringiensis* subsp. *mexicanensis*, *Bacillus thuringiensis* subsp. *morrisoni*, *Bacillus thuringiensis* subsp. *neoleonensis*, *Bacillus thuringiensis* subsp. *nigeriae*, *Bacillus thuringiensis* subsp. *ostrinae*, *Bacillus thuringiensis* subsp. *pakistani*, *Bacillus thuringiensis* subsp. *pondicheriensis*, *Bacillus thuringiensis* subsp. *shandongensis*, *Bacillus thuringiensis* subsp. *silo*, *Bacillus thuringiensis* subsp. *sotto*, *Bacillus thuringiensis* subsp. *subtoxicus*, *Bacillus thuringiensis* subsp. *tenebrionis*, *Bacillus thuringiensis* subsp. *thompsoni*, *Bacillus thuringiensis* subsp. *tochigiensis*, *Bacillus thuringiensis* subsp. *tohokuensis*, *Bacillus thuringiensis* subsp. *tolworthi*, *Bacillus thuringiensis* subsp. *toumanoffi*, *Bacillus thuringiensis* subsp. *wuhanensis*, or *Bacillus thuringiensis* subsp. *yunnanensis*. In a preferred embodiment, the biopesticide is derived from a *Bacillus thuringiensis* subsp. *kurstaki* strain. Depending on the recommended use rate for a *Bacillus thuringiensis* biopesticide formulated product, the synergist may be combined either with the *Bacillus thuringiensis* product in a tank mix or in the formulated product. In either case, the effective concentration of the applied synergist for enhancing the pesticidal activity of a commercially ineffective amount of a *Bacillus thuringiensis* biopesticide will be the same.

The *Bacillus thuringiensis* biopesticide in the pesticidal compositions of the present invention may be also derived from a cell wherein a gene, which encodes a *Bacillus thuringiensis* delta-endotoxin protein or pesticidally-active fragment thereof, has been inserted. Furthermore, within the scope of the present invention, the *Bacillus thuringiensis* biopesticide or pesticidally-active fragment thereof may be derived from a transconjugate strain wherein a plasmid containing a gene, which encodes the *Bacillus thuringiensis* delta-endotoxin protein or pesticidally-active fragment thereof, has been transferred by cell-cell conjugation.

The *Bacillus thuringiensis* biopesticide, i.e., a delta-endotoxin protein or a pesticidally-active fragment thereof, may be selected from the group including, but not limited to, CryI, CryII, CryIII, CryIV, CryV, and CryVI. More specifically, the *Bacillus thuringiensis* delta-endotoxin protein or pesticidally-active fragment thereof may include, but is not limited to, CryIA(a), CryIA(b), CryIA(c), CryIB, CryIC, CryID, CryIE, CryIF, CryIIA, CryIIB, CryIIIA, CryIIIB, CryIIIC, CryIVA, CryIVB, CryIVC, CryIVD, CryV, CryVI, and CytA. It is also within the scope of the present invention that the *Bacillus thuringiensis* biopesticide may also comprise a spore derived from the *Bacillus thuringiensis* strain. In a preferred

embodiment, the *Bacillus thuringiensis* delta-endotoxin protein is CryIA. In a most preferred embodiment, the delta-endotoxin protein is CryIA(a), CryIA(b), or CryIA(c).

The synergists of the present invention may also be used with an entomopathogenic virus alone or in combination with a *Bacillus thuringiensis* biopesticide. Examples of such entomopathogenic viruses include, but are not limited to, *Autographa californica* nuclear polyhedrosis virus (NPV), *Syngrapha falcifera* NPV, *Cydia pomonella* granulosis virus (GV), *Heliothis zea* NPV, *Lymantria dispar* NPV, *Orgyia pseudotsugata* NPV, *Spodoptera exigua* NPV, *Neodiprion lecontei* NPV, *Neodiprion sertifer* NPV, *Harrisina brillians* NPV, and *Endopiza viteana* Clemens NPV.

The synergists of the present invention may further be used with a chemical pesticide alone or in combination with a *Bacillus thuringiensis* biopesticide. Examples of such chemical pesticides include, but are not limited to, insect growth regulators, carbamates, organophosphates, pyrethroids, inorganic fluorines, pyrazoles, pyrroles, and avermectins.

The pesticidal compositions of the present invention may further comprise a deposition agent which assists in preventing the composition from drifting from the target area during application (e.g., as it is sprayed from a plane), or from being blown away from the plant once it has been deposited. In a preferred embodiment, the deposition agent in the compositions of the present invention is a proteinaceous material, which has the added benefit of being palatable to the insect. Any animal or vegetable protein is suitable for this purpose, in dry or in liquid form. Examples of useful sources of protein which can be conveniently and economically added to the composition include, but are not limited to, soy protein, potato protein, soy flour, potato flour, fish meal, bone meal, yeast extract, and blood meal. Alternative deposition agents include modified cellulose (carboxymethylcellulose), botanicals (grain flours, ground plant parts), non-phyllsilites (talc, vermiculite, diatomaceous earth), natural clays (attapulgate, bentonite, kaolinite, montmorillonite), and synthetic clays (Laponite). When utilized, the deposition agent is present in the pesticidal compositions of the present invention in an amount of between about 0.4% w/w and about 50% w/w, preferably between about 1% w/w and about 20% w/w.

The pesticidal compositions of the present invention may further comprise an antifreeze/humectant agent which suppresses the freeze point of the product and helps minimize evaporation when sprayed and which maintains deposit texture making the product more efficacious and palatable. In a specific embodiment, the antifreeze/humectant agent is

selected from the group consisting of ethylene glycol, propylene glycol, dipropylene glycol, glycerol, butylene glycols, pentylene glycols and hexylene glycols. When utilized, the antifreeze/humectant agent is present in the pesticidal compositions of the present invention in an amount of between about 0.5% w/w and about 25% w/w, preferably between about 2% w/w and about 15% w/w.

The pesticidal compositions of the present invention may further comprise a surfactant in an amount where it acts as an emulsifying, a wetting, or a dispersing agent. Examples of such surfactants are anionic surfactants such as carboxylates, for example, a metal carboxylate of a long chain fatty acid; N-acylsarcosinates; mono or di-esters of phosphoric acid with fatty alcohol ethoxylates or salts of such esters; fatty alcohol sulphates such as sodium dodecyl sulphate, sodium octadecyl sulphate or sodium cetyl sulphate; ethoxylated fatty alcohol sulphates; ethoxylated alkylphenol sulphates; lignin sulphonates; petroleum sulphonates; alkyl aryl sulphonates such as alkyl-benzene sulphonates or lower alkylnaphthalene sulphonates, e.g., butyl-naphthalene sulphonate; salts or sulphonated naphthalene-formaldehyde condensates; salts of sulphonated phenol-formaldehyde condensates; or more complex sulphonates such as amide sulphonates, e.g., the sulphonated condensation product of oleic acid and N-methyl taurine or the dialkyl sulphosuccinates, e.g., the sodium sulphonate or dioctyl succinate. Further examples of such surfactants are non-ionic surfactants such as condensation products of fatty acid esters, fatty alcohols, fatty acid amides or fatty-alkyl- or alkenyl-substituted phenols with ethylene oxide, block copolymers of ethylene oxide and propylene oxide, acetylenic glycols such as 2,4,7,9-tetraethyl-5-decyn-4,7-diol, or ethoxylated acetylenic glycols. Further examples of such surfactants are cationic surfactants such as aliphatic mono-, di-, or polyamine as acetates, naphthenates or oleates; oxygen-containing amines such as an amine oxide of polyoxyethylene alkylamine; amide-linked amines prepared by the condensation of a carboxylic acid with a di- or polyamine; or quaternary ammonium salts. When utilized, the surfactant is present in an amount of between about 0.5% w/w and about 25% w/w, preferably between about 1% w/w and about 8% w/w.

The pesticidal compositions of the present invention may further comprise an inert material. Examples of inert materials include inorganic minerals such as diatomaceous earth, kaolin, mica, gypsum, fertilizer, phyllosilicates, carbonates, sulfates, or phosphates; organic materials such as sugars, starches, or cyclodextrins; or botanical materials such as wood products, cork, powdered corncobs, rice hulls, peanut hulls, and walnut shells.

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The pesticidal compositions according to the present invention may further comprise a preservative, a feeding stimulant, an attractant, an encapsulating pesticide, a binder, a dye, a U.V. protectant, a buffer, a flow agent, or other component to facilitate product handling and application for particular target pests.

The present invention is also directed to a method for controlling pest infestation on plants comprising applying to a plant pesticidal compositions of the present invention.

The pesticidal compositions of the present invention can be applied in a dry or liquid form, e.g., a suspension, a solution, an emulsion, a dusting powder, a dispersible granule, a wettable powder, an emulsifiable concentrate, an aerosol or impregnated granule, or a concentrate or primary composition which requires dilution with a suitable quantity of water or other diluent before application. The concentrations of each component in the composition will vary depending on the *Bacillus thuringiensis* biopesticide and mode of application. The *Bacillus thuringiensis* biopesticide concentration will vary depending upon the nature of the particular composition, specifically, whether it is a concentrate or to be used directly. The composition may contain about 1% to about 98% of a solid or liquid inert carrier, about 1% to about 30% of a *Bacillus thuringiensis* biopesticide, and about 0.5% to about 25%, preferably about 1% to about 8% of the second synergist. The compositions will be preferably administered at the labeled rate for the commercial product, preferably about 0.01 pound to 5.0 pounds per acre when in dry form and at about 0.01 pint to 25 pints per acre when in liquid form.

The pesticidal compositions of the present invention can be applied directly to a plant by, for example, spraying or dusting at the time when the pest has begun to appear on the plant or before the appearance of pests as a protective measure. The pesticidal compositions can be applied by foliar, furrow, broadcast granule, "lay-by", or soil drench application. The compositions of the present invention can also be applied directly to ponds, lakes, streams, rivers, still water, and other areas subject to infestation by pests of concern to public health. The compositions can be applied by spraying, dusting, sprinkling, or the like. The spray or dust can conveniently contain another pesticide. The pesticidal compositions of the present invention are preferably applied directly to the plant.

The pesticidal compositions of the present invention can be applied to protect a number of different plant types, including, but not limited to, cereals (wheat, barley, rye,

oats, rice, sorghum and related crops), beets (sugar beet and fodder beet), drupes, pomes and soft fruit (apples, pears, plums, peaches, almonds, cherries, strawberries, raspberries, and blackberries), leguminous plants (alfalfa, beans, lentils, peas, soybeans), oil plants (rape, mustard, poppy, olives, sunflowers, coconuts, castor oil plants, cocoa beans, groundnuts), cucumber plants (cucumber, marrows, melons), fibre plants (cotton, flax, hemp, jute), citrus fruit (oranges, lemons, grapefruit, mandarins), vegetables (spinach, lettuce, asparagus, cabbages and other brassicae, carrots, onions, tomatoes, potatoes), lauraceae (avocados, cinnamon, camphor), deciduous trees and conifers (linden-trees, yew-trees, oak-trees, alders, poplars, birch-trees, firs, larches, pines), or plants such as maize, turf plants, tobacco, nuts, coffee, sugar cane, tea, vines, hops, bananas and natural rubber plants, as well as ornamentals.

The synergists of the present invention may be applied separately to a plant previously exposed to a *Bacillus thuringiensis* biopesticide.

The present invention further relates to a method for applying a synergist of the present invention to a transgenic plant, which contains a gene that encodes a *Bacillus thuringiensis* biopesticide.

The pesticidal compositions of the present invention can be used in the treatment or prevention of infestations of a number of different insect types. It is particularly preferred to use the compositions of the present invention to eliminate pests of the order Lepidoptera, e.g., *Achroia grisella*, *Acleris gloverana*, *Acleris variana*, *Adoxophyes orana*, *Agrotis ipsilon*, *Alabama argillacea*, *Alsophila pometaria*, *Amyelois transitella*, *Anagasta kuehniella*, *Anarsia lineatella*, *Anisota senatoria*, *Antheraea pernyi*, *Anticarsia gemmatalis*, *Archips* sp., *Argyrotaenia* sp., *Athetis mindara*, *Bombyx mori*, *Bucculatrix thurberiella*, *Cadra cautella*, *Choristoneura* sp., *Cochylis hospes*, *Colias eurytheme*, *Corcyra cephalonica*, *Cydia latiferreanus*, *Cydia pomonella*, *Datana integerrima*, *Dendrolimus sibericus*, *Desmia funeralis*, *Diaphania hyalinata*, *Diaphania nitidalis*, *Diatraea grandiosella*, *Diatraea saccharalis*, *Ennomos subsignaria*, *Eoreuma loftini*, *Ephestia elutella*, *Erannis tiliaria*, *Estigmene acrea*, *Eulia salubricola*, *Eupoecilia ambiguella*, *Euproctis chrysorrhoea*, *Euxoa messoria*, *Galleria mellonella*, *Grapholita molesta*, *Harrisina americana*, *Helicoverpa subflexa*, *Helicoverpa zea*, *Heliothis virescens*, *Hemileuca oliviae*, *Homoeosoma electellum*, *Hyphantria cunea*, *Keiferia lycopersicella*, *Lambdina fiscellaria fiscellaria*, *Lambdina fiscellaria lugubrosa*, *Leucoma salicis*, *Lobesia botrana*, *Loxostege sticticalis*, *Lymantria dispar*, *Macalla thyrsisalis*, *Malacosoma* sp., *Mamestra brassicae*, *Mamestra configurata*, *Manduca quinquemaculata*,

*Manduca sexta*, *Maruca testulalis*, *Melanchra picta*, *Operophtera brumata*, *Orgyia* sp.,  
*Ostrinia nubilalis*, *Paleacrita vernata*, *Papilio cresphontes*, *Pectinophora gossypiella*,  
*Phryganidia californica*, *Phyllonorycter blancardella*, *Pieris napi*, *Pieris rapae*, *Plathypena*  
*scabra*, *Platynota flouendana*, *Platynota sultana*, *Platyptilia carduidactyla*, *Plodia*  
*interpunctella*, *Plutella xylostella*, *Pontia protodice*, *Pseudaletia unipuncta*, *Pseudoplusia*  
*includens*, *Sabulodes aegrotata*, *Schizura concinna*, *Sitotroga cerealella*, *Spilonota ocellana*,  
*Spodoptera* sp., *Thaurnstopoea pityocampa*, *Tineola bisselliella*, *Trichoplusia ni*, *Udea*  
*rubigalis*, *Xylomyges curialis*, *Yponomeuta padella*; and the order Coleoptera, e.g.,  
*Leptinotarsa* sp., *Acanthoscelides obtectus*, *Callosobruchus chinensis*, *Epilachna varivestis*,  
*Pyrrhalta luteola*, *Cylas formicarius elegantulus*, *Listronotus oregonensis*, *Sitophilus* sp.,  
*Cyclocephala borealis*, *Cyclocephala immaculata*, *Macroductylus subspinosus*, *Popillia*  
*japonica*, *Rhizotrogus majalis*, *Alphitobius diaperinus*, *Palorus ratzeburgi*, *Tenebrio molitor*,  
*Tenebrio obscurus*, *Tribolium castaneum*, *Tribolium confusum*, *Tribolium destructor*.  
However, the pesticidal compositions of the present invention may also be effective against  
insect pests of the order Diptera, e.g., *Aedes* sp., *Andes vittatus*, *Anastrepha ludens*,  
*Anastrepha suspensa*, *Anopheles barberi*, *Anopheles quadrimaculatus*, *Armigeres subalbanus*,  
*Calliphora stygian*, *Calliphora vicina*, *Ceratitis capitata*, *Chironomus tentans*, *Chrysomya*  
*rufifacies*, *Cochliomyia macellaria*, *Culex* sp., *Culiseta inornata*, *Dacus oleae*, *Delia antiqua*,  
*Delia planura*, *Delia radicum*, *Drosophila melanogaster*, *Eupeodes corollae*, *Glossina austeni*,  
*Glossina brevipalpis*, *Glossina fuscipes*, *Glossina morsitans centralis*, *Glossina morsitans*  
*morsitans*, *Glossina moritans submorsitans*, *Glossina pallidipes*, *Glossina palpalis*  
*gambiensis*, *Glossina palpalis palpalis*, *Glossina tachinoides*, *Haemagogus equinus*,  
*Haematobia irritans*, *Hypoderma bovis*, *Hypoderma lineatum*, *Leucopis ninae*, *Lucilia*  
*cuprina*, *Lucilia sericata*, *Lutzomyia longipalpis*, *Lutzomyia shannoni*, *Lycoriella mali*,  
*Mayetiola destructor*, *Musca autumnalis*, *Musca domestica*, *Neobellieria* sp., *Nephrotoma*  
*suturalis*, *Ophyra aenescens*, *Phaenicia sericata*, *Phlebotomus* sp., *Phormia regina*, *Sabethes*  
*cyaneus*, *Sarcophaga bullata*, *Scatophaga stercoraria*, *Stomoxys calcitrans*, *Toxorhynchites*  
*amboinensis*, *Tripteroides bambusa*; the order Acari, e.g., *Oligonychus pratensis*, *Panonychus*  
*ulmi*, *Tetranychus urticae*; the order Hymenoptera, e.g., *Iridomyrmex humilis*, *Solenopsis*  
*invicta*; the order Isoptera, e.g., *Reticulitermes hesperus*, *Reticulitermes flavipes*, *Coptotermes*  
*formosanus*, *Zootermopsis angusticollis*, *Neotermes connexus*, *Incisitermes minor*, *Incisitermes*  
*immigrans*; the order Siphonaptera, e.g., *Ceratophyllus gallinae*, *Ceratophyllus niger*,

*Nosopsyllus fasciatus*, *Leptopsylla segnis*, *Ctenocephalides canis*, *Ctenocephalides felis*, *Echicnophaga gallinacea*, *Pulex irritans*, *Xenopsylla cheopis*, *Xenopsylla vexabilis*, *Tunga penetrans*; and the order Tylenchida, e.g., *Meloidogyne incognita*, *Pratylenchus penetrans*.

The present invention is further described by the following examples which are not construed as limiting the scope of the invention.

### Examples

#### Example 1: Cultivation of *Bacillus thuringiensis* subsp. *kurstaki* HD1

*Bacillus thuringiensis* subsp. *kurstaki* HD1 is cultivated for 72 hours at 30°C in a medium with the following composition adjusted to pH 7.0:

Corn Steep Liquor	15	g/l
Maltodextrin	40	g/l
Potato Starch	30	g/l
KH <sub>2</sub> PO <sub>4</sub>	1.8	g/l
K <sub>2</sub> HPO <sub>4</sub>	4.5	g/l

The whole broth is centrifuged to recover the crystals/spores (and other insoluble substances). The pelleted material or whole broth concentrate is used to characterize the effect of various synergists on the insecticidal activity of the crystal/spore preparation from *Bacillus thuringiensis* subsp. *kurstaki* HD1.

#### Example 2: Synergist Bioassay

The ability of a synergist to enhance the pesticidal activity of the *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate from EXAMPLE 1 is determined using a *Spodoptera exigua* bioassay. The *Spodoptera exigua* bioassay is performed in a 240 well tray where each well is filled with 0.5 ml of insect diet (10 wells for each dose). The synergist is tested in combination with three concentrations of *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate: 0.5, 0.25, and 0.12 mg per ml. The three concentrations of whole broth concentrate are also run without the synergist. The synergist is also bioassayed alone in the absence of the whole broth concentrate. Since the doses of the whole broth concentrate are chosen to be about the LC50, a score of 15/30 results when there is no synergistic effect. When fewer than 15 wells have survivors, this indicates there is synergism provided that there is no mortality in the zero dose of the whole concentrate. Some synergists

can cause stunting at the zero dose of the whole concentrate, but not cause immediate mortality. A volume of 50 µl of each concentration of diluted whole broth concentrate is first loaded into 10 wells (per dose of the whole broth concentrate), then 50 µl of the synergist solution are added to 40 wells and the mixture is allowed to dry. Once the samples are dried, 3-5 *Spodoptera exigua* eggs are placed into each well. The wells are then sealed with perforated Mylar and the trays incubated at 28°C for 7 days. After 7 days, the number of wells with survivors is determined. Stunting is rated for the zero dose of whole broth concentrate reflecting the effect of the synergist alone. In the stunt scoring system, 4=full size larvae (control larvae), 3=3/4 size of control larvae, 2=1/2 size of control larvae, 1=1/4 size of control larvae, and 0=no growth/dead.

### Example 3: Azadirachtin

Azadirachtin (MARGOSAN-O™, 0.25% azadirachtin, W.R. Grace & Co., Boca Raton, FL) is evaluated for its synergistic ability using the bioassay described in EXAMPLE 2 to enhance the pesticidal activity of the *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate of Example 1.

The results, as shown in Table 1, demonstrate that azadirachtin at a concentration of approximately 0.02 mg to 0.08 mg per mg of whole broth concentrate enhances the pesticidal activity of the *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate. When azadirachtin is assayed at the same concentration in the absence of the whole broth concentrate, azadirachtin severely stunts insect growth (stunt score of 1.5), but has no lethal effect. The total number of wells with survivors for the three doses of the whole broth concentrate in the presence of azadirachtin is 2, while in the absence of azadirachtin the total number is 23. A lethal effect by azadirachtin, however, is observed at a concentration equal to or greater than 0.1 mg/ml.

Table 1

Synergistic Additive	Bt dose (mg/ml)				Stunt Score at 0 Bt dose	Total Wells with Survivors
	0.5	0.25	0.12	0		
MARGOSAN-O™ (0.01 mg/ml)	0	0	2	10	1.5	2
No Synergist	8	7	8	10	4.0	23



#### EXAMPLE 4: Inorganic Salts Zinc Oxide and Zinc Chloride

Zinc oxide and zinc chloride (Sigma Chemical Company, St. Louis, MO) are evaluated, using the bioassay described in EXAMPLE 2, for their synergistic ability to enhance the pesticidal activity of the *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate of EXAMPLE 1.

The results, as shown in Table 2, demonstrate that zinc oxide at a concentration of approximately 10 mg to 42 mg per mg of whole broth concentrate and zinc chloride at a concentration of approximately 20 mg to 84 mg per mg of whole broth concentrate enhance the pesticidal activity of the *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate. When zinc oxide or zinc chloride is assayed at the same concentration in the absence of the whole broth concentrate, zinc oxide or zinc chloride significantly stunts insect growth (stunt scores of 1.5 and 1.6, respectively), but has essentially no lethal effect. The total number of wells with survivors for the three doses of the whole broth concentrate in the presence of zinc oxide is 4, while in the absence of the synergist the total number is 17. The total number of wells with survivors for the three doses of the whole broth concentrate in the presence of zinc chloride is 2, while in the absence of the synergist the total number is 26.

Table 2

Synergistic Additive	Bt dose (mg/ml)				Stunt Score at 0 Bt dose	Total Wells with Survivors
	0.5	0.25	0.12	0		
Zinc Oxide (5 mg/ml) no synergist	0	2	2	10	1.5	4
	4	5	8	10	4.0	17
Zinc Chloride (10 mg/ml) No Synergist	0	1	1	9	1.6	2
	10	8	8	10	4.0	26

#### EXAMPLE 5: Azadirachtin and Zinc Oxide

The combination of azadirachtin (MARGOSAN-O™) and zinc oxide (Sigma Chemical Company, St. Louis, MO) are evaluated, using the bioassay described in EXAMPLE 2, for their synergistic ability to enhance the pesticidal activity of the *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate of EXAMPLE 1. Azadirachtin and zinc oxide are run alone as controls.

The results, as shown in Table 3, demonstrate that the combination of azadirachtin and zinc oxide at concentrations of approximately 0.002 mg to 0.08 mg per mg of whole broth concentrate and approximately 2.5 mg to 42 mg per mg of whole broth concentrate, respectively, enhances the pesticidal activity of the *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate. When combining azadirachtin and zinc oxide, the concentration of each in the combination can be significantly reduced while achieving a similar synergistic effect compared to the the compounds alone at higher concentrations. When the combination of azadirachtin and zinc oxide is assayed at the same concentrations in the absence of the whole broth concentrate, the combinations produce significant stunting, but have no lethal effect.

Table 3

Synergistic Additive	Bt dose (mg/ml)				Stunt Score at 0 Bt dose	Total Wells with Survivors
	0.5	0.25	0.12	0		
MARGOSAN-OTM (0.01 mg/ml)	0	2	0	10	1.0	2
MARGOSAN-OTM (0.005 mg/ml)	0	1	1	10	1.0	2
MARGOSAN-OTM (0.001 mg/ml)	2	2	2	10	2.5	6
Zinc Oxide (5 mg/ml)	0	2	2	10	1.5	4
Zinc Oxide (2.5 mg/ml)	1	2	2	10	2.0	5
Zinc Oxide (1.25 mg/ml)	6	5	6	10	3.0	17
MARGOSAN-OTM/Zinc Oxide (0.01/5 mg/ml)	0	0	0	10	1.0	0
MARGOSAN-OTM/Zinc Oxide (0.005/5 mg/ml)	0	0	0	10	1.0	0
MARGOSAN-OTM/Zinc Oxide (0.001/5 mg/ml)	0	0	0	10	1.0	0
MARGOSAN-OTM/Zinc Oxide (0.01/2.5 mg/ml)	0	0	0	10	1.0	0
MARGOSAN-OTM/Zinc Oxide (0.005/2.5 mg/ml)	0	0	0	10	1.0	0
MARGOSAN-OTM/Zinc Oxide (0.001/2.5 mg/ml)	0	0	0	10	1.5	0
MARGOSAN-OTM/Zinc Oxide (0.01/1.25 mg/ml)	0	1	2	10	1.0	3
MARGOSAN-OTM/Zinc Oxide (0.005/1.25 mg/ml)	0	0	1	10	1.5	1
MARGOSAN-OTM/Zinc Oxide (0.001/1.25 mg/ml)	3	2	2	10	2.0	7
No Synergist	4	5	6	10	4.0	15

**EXAMPLE 6: Amphipathic Lipids**

Decanoyl-N-methyl-glucamide, digalactosyl diglyceride (in which the glycerol moiety is esterified with oleic acid and palmitic acid), dodecyl glucopyranoside, dodecyl trimethyl ammonium bromide, triolein, and phosphatidylethanolamine (Sigma Chemical

Company, St. Louis, MO) are evaluated, using the bioassay described in EXAMPLE 2, for their synergistic ability to enhance the pesticidal activity of the *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate of EXAMPLE 1.

The results, as shown in Table 4, demonstrate that these amphipathic lipids at concentrations of approximately 0.36 mg to 81 mg per mg of whole broth concentrate enhance the pesticidal activity of the *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate. When the amphipathic lipids are assayed at the same concentrations in the absence of the whole broth concentrate, no lethal or stunting effect is observed except in the case of triolein where minimal stunting is observed. The total number of wells with survivors for the three doses of the whole broth concentrate in the presence of decanoyl-N-methyl-glucamide is 5, while in the absence of decanoyl-N-methyl-glucamide the total number is 17. The total number of wells with survivors for the three doses of the whole broth concentrate in the presence of digalactosyl diglyceride is 5, while in the absence of digalactosyl diglyceride the total number is 17. The total number of wells with survivors for the three doses of the whole broth concentrate in the presence of dodecyl glucopyranoside is 7, while in the absence of dodecyl glucopyranoside the total number is 17. The total number of wells with survivors for the three doses of the whole broth concentrate in the presence of dodecyl trimethyl ammonium bromide is 6, while in the absence of dodecyl trimethyl ammonium bromide the total number is 16. The total number of wells with survivors for the three doses of the whole broth concentrate in the presence of triolein is 5, while in the absence of triolein the total number is 16. The total number of wells with survivors for the three doses of the whole broth concentrate in the presence of phosphatidylethanolamine is 7, while in the absence of phosphatidylethanolamine the total number is 17.

Table 4

Synergistic Additive	Bt dose (mg/ml)				Stunt Score at 0 Bt dose	Total Wells with Survivors
	0.5	0.25	0.12	0		
Decanoyl-N-Methyl-Glucamide (0.87 mg/ml)	1	2	2	10	4.0	5
No Synergist	5	3	9	10	4.0	17
Digalactosyl Diglyceride (0.23 mg/ml)	0	4	1	10	4.0	5
No Synergist	5	3	9	10	4.0	17
Dodecyl Glucopyranoside (0.87 mg/ml)	1	3	3	10	4.0	7
No Synergist	5	3	9	10	4.0	17
Dodecyl Trimethylammonium Bromide (0.77 mg/ml)	2	2	2	10	4.0	6
No Synergist	2	7	7	10	4.0	16
Triolein (9.74 mg/ml)	0	1	4	9	3.0	5
No Synergist	2	7	7	10	4.0	16
Phosphatidylethanolamine (0.18 mg/ml)	0	2	5	10	4.0	7
No Synergist	5	3	9	10	4.0	17

**EXAMPLE 7: Analgesics**

Acetyl salicylic acid, ibuprofen, and acetaminophen (Sigma Chemical Company, St. Louis, MO) are evaluated, using the bioassay described in EXAMPLE 2, for their synergistic ability to enhance the pesticidal activity of *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate of EXAMPLE 1.

The results, as shown in Table 5, demonstrate that these compounds at concentrations in the range of 7.56 mg to 86 mg per mg of whole broth concentrate enhance the pesticidal activity of the *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate. When each of these compounds is assayed at the same concentration in the absence of the whole broth concentrate, no lethal or stunting effect is observed. The total number of wells with survivors for the three doses of the whole broth concentrate in the presence of acetyl salicylic acid is 2, while in the absence of acetyl salicylic acid the total number is 16. The total number of wells with survivors for the three doses of the whole broth concentrate in the presence of acetaminophen is 7, while in the absence of acetaminophen the total number is



#### EXAMPLE 9: Poly-L-Lysine

Poly-L-lysine (Sigma Chemical Company, St. Louis, MO) is evaluated, using the bioassay described in EXAMPLE 2, for its synergistic ability to enhance the pesticidal activity of *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate of EXAMPLE 1.

The results, as shown in Table 7, demonstrate that poly-L-lysine at a concentration of approximately 5 mg to 83 mg per mg of whole broth concentrate enhances the pesticidal activity of the *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate. When poly-L-lysine is assayed at the same concentration in the absence of the whole broth concentrate, it minimally stunts insect growth (stunt score of 3.0), but has no lethal effect. The total number of wells with survivors for the three doses of the whole broth concentrate in the presence of poly-L-lysine is 5, while in the absence of poly-L-lysine the total number is 13.

Table 7

Synergistic Additive	Bt dose (mg/ml)				Stunt Score at 0 Bt dose	Total Wells with Survivors
	0.5	0.25	0.12	0		
Poly-L-Lysine (2.5 - 10 mg/ml)	2	2	1	10	3.0	5
No Synergist	5	4	4	10	4.0	13

#### EXAMPLE 10: Alpha-Tocopherol

Alpha-tocopherol (Sigma Chemical Company, St. Louis, MO) is evaluated, using the bioassay described in EXAMPLE 2, for its synergistic ability to enhance the pesticidal activity of *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate of EXAMPLE 1.

The results, as shown in Table 8, demonstrate that alpha-tocopherol at a concentration of approximately 17 mg to 72 mg per mg of whole broth concentrate enhances the pesticidal activity of the *Bacillus thuringiensis* subsp. *kurstaki* whole broth concentrate. When alpha-tocopherol is assayed at the same concentration in the absence of the whole broth concentrate, it minimally stunts insect growth (stunt score of 3.5), but has no lethal effect. The total number of wells with survivors for the three doses of the whole broth concentrate in the presence of alpha-tocopherol is 2, while in the absence of alpha-tocopherol the total

number is 16. A lethal effect by alpha-tocopherol, however, is observed at a concentration equal to or greater than 50 mM.

Table 8

Synergistic Additive	Bt dose (mg/ml)				Stunt Score at 0 Bt dose	Total Wells with Survivors
	0.5	0.25	0.12	0		
Alpha-Tocopherol (8.61 mg/ml)	0	1	1	10	3.5	2
No Synergist	4	6	6	10	4.0	16

The invention described and claimed herein is not to be limited in scope by the specific embodiments herein disclosed, since these embodiments are intended as illustrations of several aspects of the invention. Any equivalent embodiments are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.



What is claimed:

1. A pesticidal composition comprising an amount of a *Bacillus thuringiensis* biopesticide and a synergist in an effective amount for enhancing the pesticidal activity of the *Bacillus thuringiensis* biopesticide to effectively control a pest wherein the synergist is selected from the group consisting of azadirachtin, zinc oxide, zinc chloride, a combination of azadirachtin and a zinc salt, decanoyl-N-methyl-glucamide, digalactosyl diglyceride, dodecyl glucopyranoside, dodecyl trimethyl ammonium bromide, triolein, phosphatidylethanolamine, acetylsalicylic acid, ibuprofen, acetaminophen, esculin, poly-L-lysine, and alpha-tocopherol.
2. The pesticidal composition according to Claim 1, wherein said amount of said *Bacillus thuringiensis* biopesticide alone is ineffective for controlling said pest.
3. The pesticidal composition according to Claim 1, wherein said *Bacillus thuringiensis* biopesticide is a delta-endotoxin protein or a pesticidally-active fragment thereof.
4. The pesticidal composition according to Claim 3, wherein said delta-endotoxin protein is a *Bacillus thuringiensis* subsp. *kurstaki* delta-endotoxin protein or a pesticidally-active fragment thereof.
5. The pesticidal composition according to Claim 4, wherein said *Bacillus thuringiensis* subsp. *kurstaki* delta-endotoxin protein is a CryIA protein or a pesticidally-active fragment thereof.
6. The pesticidal composition according to Claim 1, wherein said synergist is present in an amount in the range of between about 0.001 gram and about 100 gram per gram of said *Bacillus thuringiensis* biopesticide.
7. The pesticidal composition according to Claim 6, wherein said synergist is present in an amount in the range of between about 0.001 gram and about 25 gram per gram of said *Bacillus thuringiensis* biopesticide.

8. The pesticidal composition according to Claim 1, wherein said synergist is an azadirachtin.
9. The pesticidal composition according to Claim 1, wherein said synergist is zinc oxide or zinc chloride.
10. The pesticidal composition according to Claim 1, wherein said synergist is a combination of azadirachtin and a zinc salt.
11. The pesticidal composition according to Claim 10, wherein said zinc salt is selected from the group consisting of zinc oxide, zinc chloride, and zinc sulfate.
12. The pesticidal composition according to Claim 1, wherein said synergist is an amphiphatic lipid.
13. The pesticidal composition according to Claim 12, wherein said amphiphatic lipid is selected from the group consisting of decanoyl-N-methyl-glucamide, digalactosyl diglyceride, dodecyl glucopyranoside, dodecyl trimethyl ammonium bromide, triolein, and phosphatidylethanolamine.
14. The pesticidal composition according to Claim 1, wherein said synergist is an analgesic compound.
15. The pesticidal composition according to Claim 14, wherein said analgesic compound is selected from the group consisting of acetylsalicylic acid, ibuprofen, and acetaminophen.
16. The pesticidal composition according to Claim 1, wherein said synergist is esculin.
17. The pesticidal composition according to Claim 1, wherein said synergist is poly-L-lysine.
18. The pesticidal composition according to Claim 1, wherein said synergist is alpha-tocopherol.

19. A method for controlling a pest comprising exposing said pest to a pesticidal composition according to claim 1.

20. A method for controlling a pest comprising applying to a transgenic plant, which contains a gene that encodes a *Bacillus thuringiensis* biopesticide, a synergist in an effective amount for enhancing the pesticidal activity of the *Bacillus thuringiensis* biopesticide to effectively control a pest wherein said synergist is selected from the group consisting of azadirachtin, zinc oxide, zinc chloride, a combination of azadirachtin and a zinc salt, decanoyl-N-methyl-glucamide, digalactosyl diglyceride, dodecyl glucopyranoside, dodecyl trimethyl ammonium bromide, triolein, phosphatidylethanolamine, acetylsalicylic acid, ibuprofen, acetaminophen, esculin, poly-L-lysine, and alpha-tocopherol.